

P.C. 2108.91

PATENT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

In re application of
LAWRENCE A. JOHNSON
Method to Preselect the
Sex of Offspring
Serial No. 07/692,958
Filed April 26, 1991

Group Art Unit 188
Examiners J. Witz &
D. W. Robinson

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JUL 15 1992

cc: (w/encls.)
OGC (w/o ref)
Johnson Davies
Oliff NCAUR

ASST COMR PATENTS

ARS:NCAUR:CPR:cpr:kam 2108pet

The Honorable

The Commissioner of Patents & Trademarks

Sir:

PETITION UNDER 37 CFR 1.313(a)
WITHDRAWAL FROM ISSUE

Applicant respectfully requests that the above-identified application be withdrawn from issue. THE COMMISSIONER IS AUTHORIZED TO CHARGE DEPOSIT ACCOUNT 01-0455 IN THE AMOUNT OF \$130.00 FOR THE REQUIRED FEE UNDER 37 CFR 1.17(i)(1).

The Notice of Allowability in the above-identified application was received on March 30, 1992, and the issue fee was promptly paid on April 1. Withdrawal from issue is requested for the following reasons:

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on April 29, 1992.
(Date of Deposit)

Curtis P. Ribando

Name of Applicant, assignee, or
Registered Representative

Signature

Date of Signature

- 1 -

Serial No. 07/692,958

In the Examiner's Statement of Reasons for Allowance, the Examiner stated:

The Declarations filed January 13, 1992 and May 30, 1991 combined serve to indicate that the sorting of intact sperm with tails combined with the staining at a temperature of 30-39°C patentably the [sic] defines over the prior art which shows sorting of tailless sperm heads having been stained at room temperature.

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On April 14, 1992, the undersigned received a copy of the Supplementary European Search Report from counsel handling matters relating to the foreign patent prosecution. By virtue of that report, applicant's counsel first became aware of the Adair reference cited on the PTO-1449, submitted herewith. Similar to the applicant's invention, Adair relates to separating sperm into X and Y components so that the sex of offspring can be predetermined. The reference relies upon the principal of differential DNA staining of X and Y sperm cells with a fluorescent dye and separating based on the difference in fluorescence. Of particular significance is the passage on page 5, lines 24-29, wherein it is stated:

The semen is then stored in a water bath at approximately the body temperature of the animal. This is between about 35°C and about 38.5°C, but is usually about 37°C. If the temperature is substantially above or below this range, sperm mobility will decrease and the survival rate of the sperm will decrease below acceptable limits.

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At first blush, the temperature treatment of Adair could be construed as suggesting the applicant's point of novelty. Therefore, applicant and the undersigned wish to fulfill their duty of disclosure under 37 CFR 1.156 by submitting this reference for consideration by the Examiner.

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? Applicant also wishes to resolve herein any potential issues of patentability which could be raised by this reference. Stated below are the reasons why Adair, taken alone or in combination with the art of record, fails to teach or suggest the claimed invention.

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Though Adair makes it clear that the collected semen should be stored at the stated mammalian body temperature range prior to staining, nowhere is it stated that the semen should be held at that temperature during the staining. As an alternative to storing the semen at body temperature, the sperm cells can be separated out of the semen fluid and diluted in a suitable saline solution (presumably at room temperature; see page 3, lines 25-28). There is no clear suggestion by Adair that the temperature range claimed by the applicant or that taught by Adair would be instrumental in facilitating absorption of the dye. The saline solution alternative would support the supposition that Adair failed to recognize any correlation between the holding temperature and successful dyeing of the cells. In fact, Adair relies upon a completely different method to



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promote dye uptake. As noted on page 5, lines 30-34, and on page 6, lines 15, a cell membrane diffusion material must be used for this purpose for most mammalian sperm. The use of the cell diffusion material is further discussed on page 2, lines 11-20, of the reference.


The Examiner will note that the passage from Adair reproduced above speaks to effects of temperature on the viability of the sperm, but makes no mention of the effects of time. The implication of this passage and that on page 3, lines 25-28, is that the sperm cells will remain viable indefinitely at the designated temperatures. The person in the art of the biological sciences will recognize that many cells, microorganisms, enzymes, and the like are long-lived within the range of 15°-38.5° C. But as the data and statements of record in the application will show, sperm cells quickly lose viability at those temperatures. Adair fails to appreciate this fact and also fails to take any precautions to avoid sperm devitalization. No actual viability data is presented. In contrast, all the applicant's allowed claims require that the incubation take place "for a period of time sufficiently long for staining to take place uniformly but sufficiently short to preserve viability of the sperm." Adair's only concern about sperm viability relates to using too high of a diffusion material concentration (page 6, lines 11-15) or too high of a dye concentration (page 6, lines 28-30).

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Finally Adair is silent regarding whether or not his method preserves the integrity of the sperm cells and whether or not his process is operable for separating intact sperm with tails. This reference is marked by many of the same deficiencies as the art previously of record. Upon careful review, it becomes readily apparent that Adair neither anticipates nor renders obvious the claimed invention.

For the above-stated reasons, the Examiner is asked to reaffirm the patentability of Claims 9-34 and to pass the case to issue.

Respectfully submitted,


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720-4866 or 720-2421

Enclosures:
Supplemental List of Prior Art Cited by Applicant
Reference AL

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CORRESPONDENCE RECEIPT

P.C. 2108.91-Lawrence A. Johnson
Serial No. 07/692,958-filed 4/26/91

Petition Under 37 CFR 1.313(a) WITHDRAWAL FROM ISSUE^{Ed}
(6 pp.) w/Reference AL



cm 4/29/92

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ISSUE NOTIFICATION



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	PATENT NUMBER	ISSUE DATE	ATTORNEY DOCKET NO.
07/692,958	5135759	08/04/92	2108.91

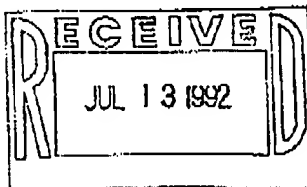
5759
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APPLICANT(S) LAWRENCE A. JOHNSON, SILVER SPRING MARYLAND

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ISSUANCE

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TOTAL P.09

FORM PTO-1447 (REV. 7-82)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTY. DOCKET NO. P.C. 2108.91		SERIAL NO. 07/692,958	
Supplemental / LIST OF PRIOR ART CITED BY APPLICANT (Use several sheets if necessary)				APPLICANT Lawrence A. Johnson		GROUP J. Witz &	
				FILING DATE 4/26/91		188: D. W. Robinson	
U.S. PATENT DOCUMENTS							
EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
	AA						
	AB						
	AC						
	AD						
	AE						
	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						
FOREIGN PATENT DOCUMENTS							
		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES NO
	AL	84/01265	12/84	PCT/US			X
	AM						
	AN						
	AO						
	AP						
OTHER PRIOR ART (Including Author, Title, Date, Pertinent Pages, Etc.)							
	AR						
	AS						
	AT						
EXAMINER				DATE CONSIDERED			
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.							



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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification³ : A01N 1/02, 435/2 A61K 35/48, 424/105	A1	(11) International Publication Number: WO 84/ 01265 (43) International Publication Date: 12 April 1984 (12.04.84)
<div data-bbox="178 441 852 934">(21) International Application Number: PCT/US82/01478 (22) International Filing Date: 5 October 1982 (05.10.82) (71) Applicant: GENETIC ENGINEERING, INC. [US/US]; 136 Avenue and North Washington Street, P.O. Box 3354, Denver, CO 80233 (US). (72) Inventor: ADAIR, Edwin ; 2800 S. University Blvd., #97 Guard House, Denver, CO 80210 (US). (74) Agent: TOBIA, Annette; 56 Battle Road, Princeton, NJ 08540 (US). (81) Designated States: CH (European patent), DE (European patent), FR (European patent), GB (European patent), JP. Published <i>With international search report.</i></div> <div data-bbox="1177 756 1485 840">- A01N1/02</div>		

DOC

(54) Title: METHOD OF TREATING COLLECTED MAMMAL SEMEN AND SEPARATING SPERM INTO X AND Y COMPONENTS

(57) Abstract

A method of sexing viable sperm cells using a cell cytometer in combination with selective labelling of sperm cells with fluorescent dye. Upon excitation of the selectively labelled X and Y chromosome bearing cells a detection system is activated so that X and Y sperm cells are channeled into two separate collection ports.

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Method of Treating Collected Mammal Semen
and Separating Sperm Into X and Y Components

Technical Field

05 This invention relates to an improved method of preserving
collected mammalian semen and separating the sperm contained
therein into X and Y components so that a female of the species
may be artificially inseminated with separated sperm to produce
10 offspring of a desired and predetermined sex. This invention is
particularly useful for preserving cattle semen and separating it so
that sperm bearing only X chromosomes can be separated and used
to produce dairy herds and sperm bearing only Y chromosomes can
be used to produce beef herds. This invention also has usage in
breeding horses and other animals.

Background Art

15 Until now, no known way has been suggested in the prior art
for obtaining large populations of viable sexed sperm. This is of
particular significance in cattle breeding wherein it is desired to
have a large number of female offspring when breeding dairy cattle
and a large number of male offspring when breeding beef cattle.
20 Also, such a sexing technique, if available, would have wide
application in many situations for example involving humans where
predetermination of sex of offspring would be desirable.

Some physical characteristics of sperm that are of potential
use in separating sperm cells are: higher density or packing of
25 the Y chromosome, greater DNA content of the X chromosome,
slightly larger size and heavier weight of sperm containing the X
chromosome; generally higher velocity of movement of the Y
chromosome containing sperm; and an apparent dense negative
electrical surface charge on the X chromosome. However, the
30 methods of separation of sperm utilizing these physical properties
have, for the most part, been only partially successful, with
separation percentages ranging from 60% to 80%. Thus, there is a

very great need for a method of sperm separation which is virtually 100% accurate and which can be done very rapidly. Furthermore, in order to accomplish separation, it is necessary to maintain or get the semen which has been collected in a fluid non-coagulated condition before the separation of the sperm can be accomplished.

It is well known that in primates, such as humans and gorillas, the Y chromosome of sperm tends to fluoresce to a special brightness when stained with a dye such as quinacrine or quinacrine mustard whereas the X chromosome of sperm will not so fluoresce. It is also known that the sperm of most other animals will not normally be subject to this selective staining unless a cell membrane diffusion material or agent, such as an appropriate enzyme or chemical, is used to facilitate passage through the cell membrane so that the dye can enter the cell. A commercially available chemical for this purpose is dimethylsulfoxide. It is also known that by adding papaya protease to the sperm of horses and bulls, the cell membrane can be penetrated by the dye so that selective identification of the X and Y chromosomes can be accomplished under a microscope. A disclosure of this technique is found in U.S. Patent No. 4,155,831 entitled "Thermal Convection Counter Streaming Sedimentation and Forced Convection Galvanization Method and Apparatus for Controlling the Sex of Mammalian Offspring," issued May 22, 1979 to Bhairab C. Bhattacharya. Such a process has been used in the prior art to determine the success ratio in separating X and Y chromosome bearing sperm, hereinafter X and Y sperm, by other known methods.

A device for identifying and then separating live blood cells from dead blood cells is disclosed in U.S. Patent No. 3,791,517 for "Digital Fluid Amplifier Particle Sorter," issued February 12, 1974 to Mitchell Friedman. It is suggested in this patent that the separation of sperm cells be done, by use of selective fluorescent radiation responses from X and Y sperm labelled differentially with a fluorescent dye.

Disclosure of Invention

In accordance with this invention, a method of separating viable sperm cells according to whether they carry an X chromosome or a Y chromosome is described. The separated
05 batches of cells are suitable for use in artificial insemination of humans, cows and other livestock animals so that offspring of predetermined sex are obtained.

The method of separation, it is hypothesized, depends on basic differences in X and Y sperm cells. For example, each X
10 sperm has a greater DNA content than a corresponding Y sperm since the X chromosome has a greater DNA content than a comparable Y chromosome. Hence, it is postulated that the X sperm should incorporate greater absolute amounts of certain dyes and other labels than Y sperm. On the other hand, it is believed
15 that the Y chromosome of Y sperm is more dense or tightly packed than a corresponding X chromosome. Hence it is postulated some dyes and other label should become incorporated or associated with the Y chromosome in a denser fashion than they do for a comparable X chromosome and will be perceived accordingly.

20 Semen which contains sperm is collected from a mammal and treated by adding an anti-coagulant, such as mammal saliva, to prevent coagulation and hasten liquefaction. Any anticoagulant that does not affect viability will work. In particular, human saliva has been found very satisfactory for this purpose and is
25 readily available. The semen is then diluted with a diluting solution and stored in a water bath at substantially the body temperature of the mammal or the sperm cells are centrifuged out of the semen fluid and diluted in a suitable saline solution.

When the semen is of mammals other than primates and voles,
30 a cell membrane diffusion material, such as sperm-free human semen, can be added to the mammalian semen so that a fluorescent dye can penetrate the cell membrane of the sperm cell and become localized around the chromosomes therein. The dye will penetrate the cell membranes of the sperm cell of primates and vole semen
35 without prior preparation.

The selection of dye depends on the method of detection used to select the X and Y sperm. If a scanning procedure is used wherein any bright spots of fluorescent dye would be detected, such as that which it is postulated characterize the Y chromosome because of its higher density than an ideal dye is quinacrine or quinacrine mustard.

However, if the total fluorescence per sperm cell is being detected then a more suitable dye has been found to be acridine, acridine orange and derivatives thereof such as ethidium, bromide, mithramycin or any combination thereof. Another suitable dye is DAPI (4, 6-diamidino-2-phenylindole).

Following staining with the dye the semen is put into a detection chamber. The semen is flowed through the chamber so that ideally only one sperm cell at a time is irradiated with ultraviolet light. The light causes the dye to fluorescence. A detection device in the chamber senses the degree of fluorescence and provides an output signal in response to the sensed cell which varies according to the degree of fluorescence. The stream then passes through a fluidic amplifier and the stream is switched in response to each sensed cell in the detection chamber to direct the sensed cell to one of two outlet ports. If the detection device is programmed to scanning the cell for bright spots the Y sperm will trigger the system presumably because of the higher density of the dye localized around the denser Y chromosome. If instead the greater absolute degree of fluorescence is being detected, the X sperm will trigger the system presumably because the X sperm contains more DNA; and, hence more dye is localized around it. In any way, the X and Y chromosomes can be effectively separated into separate containers for use in artificial insemination of female animals in order to produce the desired sexed offspring.

It will be apparent that this method of treating mammalian semen and separating X and Y sperm is rapid and highly efficient.

Additional advantages of the invention will become apparent from the detailed description which follows.

Best Mode for Carrying Out the Invention.

05 Semen is first collected from a mammal in any manner which is known to one skilled in the art. For example, with cattle and horses, teaser animals or electrical stimulation may be used. The various methods of collecting semen from animals is explained in conventional veterinarian reference texts.

10 The collected semen is placed in a clean, sterile container and may be treated with an enzyme solution to hasten liquefaction. Cattle semen for example is highly viscous and will not flow in a stream as necessary for separation unless an anti-coagulant is added and a dilutant is also added. Advantageously, it has been found that one of the best enzyme solutions and one of the most physiologically compatible is mammalian saliva, and particularly human saliva. In practice, about 1 ml of human saliva is added to 15 each quantity of about 2.0 ml to about 3.5 ml of semen. If the quantity of human saliva added is significantly less than 1 ml, the reaction will be too slow. However, an amount larger than 1 ml will have no adverse effect, but is unnecessary.

20 The semen is then diluted with any known commercially available semen diluting solution on about a 1:1 basis. Commercially available semen diluting solutions contain egg yolk, glycerol, glucose and citrate. Preparation of these solutions is set forth in Fertility and Sterility by Berman and Sasada, published in 1966. One such anti-coagulant is alpha-amylase. The semen is then 25 stored in a water bath at approximately the body temperature of the animal. This is between about 35°C and about 38.5°C, but is usually about 37°C. If the temperature is substantially above or below this range, sperm mobility will decrease and the survival rate of the sperm will decrease below acceptable limits.

30 When it is time to selectively identify and separate the X and Y sperms and the mammal is not a primate or a vole, a cell membrane diffusion material must be added to the collected semen so that the dye can penetrate the cell. The dye will penetrate in primate and vole semen without further treatment. One cell

membrane diffusion material which has been found satisfactory is DMSO, commonly known as dimethylsulfoxide and sometimes referred to as disulfomethoxide. It has been found that the addition of about 0.05 ml of DMSO to a quantity of about 2.0 ml to about 3.5 ml of semen provides sufficient penetration of the cell membrane by the dye.

Another cell membrane diffusion material which has been found quite satisfactory is sperm-free human semen. The sperm-free human semen is added in a quantity of about 0.5 ml human semen to a quantity of about 2.0 ml to about 3.5 ml of mammal semen. If either DMSO or human semen is added in quantities substantially below the indicated ranges, the diffusion of the dye will be very slow whereas if the amounts added are substantially above the indicated ranges, the DMSO and human semen will have a toxic effect and kill substantial numbers of sperm.

The dyes which has been found most satisfactory are taken from the group consisting of quinicrine, quinicrine hydrochloride, quinicrine dihydrochloride and quinicrine mustard. When quinicrine hydrochloride or quinicrine dihydrochloride is used, it is diluted in a solution of ion-free distilled water to a strength in the range of about 0.05% to about 5.0%. The most satisfactory range has been found to be between about 0.5% and about 1.0% in solution. When quinicrine mustard is used, it is diluted with ion-free distilled water to a solution having a strength in the range of about 0.001% to about 0.01% whereas the optimum range is about 0.005% solution. If the solutions are substantially below the indicated range in concentration, the dye will take too long to stain, whereas if the solutions are substantially above the indicated ranges in concentration, the dye will have a toxic effect and kill substantial numbers of the sperm. The dye solution is put in contact for a period of at least several minutes, but usually for a period of at least 15 minutes to assure good dye penetration. Confirmation of staining is obtained by separating a small part of the specimen and checking for staining under a fluorescent microscope.

After the semen has been thus treated, it can be fed through a digital fluidic amplifier particle sorter such as that described in the above-mentioned U.S. Patent No. 3,791,517 to Friedman. This device is sold under the trademark "CYTOFLUOROGRAF" by Ortho Instruments, Inc. of Westwood, MA, U.S.A. The disclosure of this patent is incorporated herein by reference. By using this apparatus, the difference in fluorescence of X and Y chromosome bearing cells entrained in a stream of diluted semen is detected and the resultant different signals which are produced as a result of this detection are used to control a fluidic amplifier located downstream to switch the X sperm to one outlet port and the Y sperm to another outlet port so they can be separated and collected.

In particular, the semen is fed in a very narrow stream so that the sperm cells in the semen move essentially single file past a laser light beam which is provided with an ultraviolet filter. The laser projects a very narrow beam in which a pattern of illumination of the beam, when it strikes the sperm cell, appears as a thin line of light transverse to the stream of cells. Electrical photoresponsive pick-up elements are arranged around the outside of the detection chamber through which the stream passes to detect any scattering of light due to the ultraviolet light striking a fluorescent Y chromosome. The excitation wavelength of the laser beam is in the range of 457 nm to 488 nm. The emission wavelength from the fluorescent Y sperm will be around 351 nm. The fluid amplifier includes a transducer which is responsive to any scattering of light due to fluorescence of a Y sperm which it is postulated will create a turbulence in the stream to cause a "wall attachment effect" as is well known in fluidics. Thus, any X chromosome it is postulated will pass along the stream uninterrupted and out a first outlet port whereas the Y chromosomes will be diverted due to reaction of the transducer to the scattered light causing them to be diverted and pass through a second outlet port. By this means, the semen can be separated into X and Y sperm and separately collected for

artificial insemination of an animal at a future time wherein the sex of the offspring will be predetermined. The separation accuracy of this method is extremely high compared with the 60% to 80% separation possible with other techniques. In practice, using this technique, the separation effectiveness is nearly 100% and is at least greater than 90%. After separation, the sperm may be frozen for use at a future time in a manner which is well known.

From the foregoing, it can be seen that a highly novel and efficient method has been provided for preparing mammal semen for separating the X and Y sperm so that a female animal such as a cow or horse can be artificially inseminated and the sex of the offspring predetermined. Of course, it will be understood that the use of this technique would have application with respect to any mammal.

The invention has been described in detail with particular reference to a preferred embodiment thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

SUBSTITUTE SHEET

BURE
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Claims

What is claimed is:

1. A method of preserving semen for use in artificial insemination comprising the steps of:
 - 05 collecting semen from a mammal;
 - treating the mammal semen by adding mammal saliva to prevent coagulation;
 - storing the semen in a water bath at substantially the body temperature of the mammal.
- 10 2. The method, as claimed in Claim 1, wherein:
 - human saliva is added to the mammal semen to prevent coagulation.
3. The method, as claimed in Claim 1, wherein:
 - a quantity of about 1.0 ml of human saliva is added to a
 - 15 quantity of about 2.0 ml to about 3.5 ml of mammal semen.
4. The method, as claimed in Claim 1, wherein:
 - the mammal semen is diluted on about a 1:1 ratio.
5. The method, as claimed in Claim 1, wherein:
 - the treated and diluted mammal semen is stored at a
 - 20 temperature in the range of about 35°C to about 38.5°C.
6. The method, as claimed in Claim 4, wherein:
 - the treated and diluted semen is stored at a temperature of about 37°C.
7. A method of separating viable X and Y chromosomes
 - 25 bearing sperm cells comprising the steps of:
 - (a) selectively staining the chromosomes of the sperm cells with a flurescent dye;
 - (b) flowing the stained sperm cells, single file, through a flow cytometer comprising a detection
 - 30 chamber;
 - (c) irradiating said sperm cells with ultraviolet light;
 - (d) detecting fluorescence;
 - (e) translating the detected fluorescence into an output signal;

- (f) switching the flow in response to the output signal;
- (g) collecting the sexed sperm.

8. The method, as defined in Claim 7 wherein the detection chamber detects total DNA fluorescence.

05 9. The method as defined in Claim 8 wherein the fluorescent dye is selected from the group consisting of acridine, acridine orange and derivatives thereof.

10. The method as defined in Claim 9 wherein the dye is ethidium bromide and mithramycin.

10 11. The method as defined in Claim 7 wherein the detection chamber detects the fluorescence of the Y chromosome.

12. The method as defined in Claim 11 wherein the fluorescent dye is selected from the group consisting of quinicrine, quinicrine hydrochloride, quinicrine dihydrochloride, and quinicrine mustard.

13. The method, as claimed in Claim 12, wherein, the dye is a quinicrine solution having a strength of about 0.05% to about 5.0% of quinicrine hydrochloride and quinicrine dihydrochloride.

20 14. The method, as claimed in Claim 13, wherein, said solution has a strength of about 0.5% to about 1.0%.

15. The method, as claimed in Claim 11, wherein, the dye is a quinicrine mustard solution having a strength of about 0.001% to about 0.01% or quinicrine mustard.

25 16. The method, as claimed in Claim 15, wherein, said solution has strength of about 0.005%.

17. The method, as claimed in Claim 11, wherein, said semen is irradiated with ultraviolet light having a wavelength between about 457 nm and about 488 nm.

30 18. A method of separating X and Y sperm in mammal semen comprising the steps of:

collecting semen from the mammal;

adding an anticoagulant to prevent coagulation;

diluting the semen;

35 maintaining the temperature of the treated and diluted semen at a temperature of about 37°C;

adding a fluorescent dye to the semen to cause the Y chromosome to specially fluoresce under ultraviolet light;

irradiating the semen with ultraviolet light;

05 moving the semen in a stream through a detection chamber;

sensing the presence of the specially fluorescent Y chromosome in the detection chamber;

providing an output signal in response to the sensed chromosome;

10 passing the stream from the detection chamber through a fluidic amplifier; and

switching the stream in response to the Y chromosome to direct the Y containing sperm cell to one of two outlet ports, and the X chromosome containing sperm cell to the other outlet port
15 collecting the sexed sperm.

19. The method, as claimed in Claim 18 wherein,
about 0.05 ml of DMSO is added to about 2.0 ml to about 3.5 ml of semen as the cell membrane diffusion material.

20. The method, as claimed in Claim 18, wherein,
20 about 0.5 ml of sperm-free human semen is added to about 2.0 ml to about 3.5 ml of semen as the cell membrane diffusion material.

INTERNATIONAL SEARCH REPORT

International Application No. **PCT/US82/01478**

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC ?

A01N 1/02	435/2
A61K 35/48	424/105

II. FIELDS SEARCHED

Minimum Documentation Searched *

Classification System	Classification Symbols
U.S.	435/2 424/105 436/63, 906
	209/577, 580, 587

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched *

Chemical Abstracts 8th Collective to date
"semen", "sperm", "saliva"

III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴

Category *	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
A	US, A, 3,185,623 Published 25 May 1965, Smith et al	1-6
X	US, A, 3,586,859 Published 22 June 1971, Katz et al	7-20
A	US, A, 3,816,249 Published 11 June 1974, Bhattacharya	1-6
A	US, A, 4,092,229 Published 30 May 1978, Bhattacharya	7-20
X	US, A, 3,207,910 Published 21 September 1965 Hirschfeld et al	7-20
X	US, A, 3,305,089 Published 21 February 1967 Fraenkel	7-20

* Special categories of cited documents: ¹⁶

"A" document defining the general state of the art
 "E" earlier document but published on or after the international filing date
 "L" document cited for special reason other than those referred to in the other categories
 "O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but on or after the priority date claimed
 "T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention
 "X" document of particular relevance

IV. CERTIFICATION

Date of the Actual Completion of the International Search *

15 February 1983

International Searching Authority *

ISA/US

Date of Mailing of this International Search Report *

02 MAR 1983

Signature of Authorized Officer ²⁰

Sam Rosen

SAM ROSEN
EXAMINER